GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
- *Choose File, Save As*
- *Select No Compression in the save options.*
- *Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.*
- *Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.*

These instructions are very generic. The menu options for your graphics program may be different.

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Strain Name **Ascl1-TdTomato2**	MMRRC Stock Number **43552**	

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NAME OF PCR: **Ascl1-TdTomato** **MMRRC:** **43552**

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	6.9
10x Buffer	1.2
Loading buffer(10x Sucrose+dye)	1.2
	.24
Primer 1. (stock concentration is 20µM)	.12
Primer 2. (stock concentration is 20µM)	.12
Primer 3. (stock concentration is 20µM)	.12
Taq Polymerase 5Units/µL	.1
Earpunch DNA extracted w/ NaOH at 95C for 1 hour, quenched in Tris buffer	2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**12 µL**

Comments on protocol:

- Hotstart not necessary but helpful.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	98	2:00	**1**
2. Denaturation		98	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	60	0:30	35**x**
4. Elongation		72	0:30	
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. WT_F	AACTGATGCGCTGCAAACG
2. Com_R	CCATCCCCACATGAAGCGTA
3. Mut_F	AGGATCCATGAATGGGCACG
4.	
5.	

Electrophoresis Protocol:

Argarose: 1.5% V:110

Estimated Running:Time: 25 min.

Primer Combination	Band	Genotype
WT_F/Com_R	489 bp	WT
Mut_F/Com_R	707 bp	Mut
	bp	

Please size gel images to fit in this space

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Protocol / Gel Comments:

Gel is 12 ul of total reaction loaded on a 1.5% gel. Ladder is 2-Log ladder from NEB. Lanes are Wildtype, homozygous, heterozygous (94, 99, 165), respectively.

Gel pictures: